Drug Delivery by Controlling a Supramolecular Host–Guest Assembly with a Reversible Photoswitch

Nuno Basílio*[a] and Uwe Pischel*[b]

Abstract: The reversibly switchable trans-chalcone/flavylium photochromic system was successfully coupled to the complexation equilibrium of a drug-cucurbit[7]uril host–guest assembly. Hence, the phototriggered release of memantine under illumination at 366 nm was observed. The process can be partially reverted through a thermally activated back reaction.

The assembly and disassembly of host–guest complexes by means of the application of external stimuli is an elegant exercise of functional supramolecular chemistry that builds on the reversibility of non-covalent interactions.[1–5] This has led to viable approaches towards intelligent supramolecular materials, molecular information processing, the release/capture of compounds in bio-inspired applications, and drug-delivery systems.[6–11] Among other stimuli, such as chemical and electrochemical signals, the use of light has been a preferred choice due to the possibility of conducting spatiotemporally and remotely controlled experiments.[1, 12–16] Our own efforts in this field have focused on supramolecular complexes with cucurbituril hosts and their manipulation by means of photoinduced pH jumps.[17, 18] The choice of cucurbiturils as host macrocycles for these applications is motivated by their unique supramolecular chemistry, including extraordinary high binding constants for a variety of organic guests in water.[3, 19–22] As a consequence, the nanotechnological application potential of cucurbiturils at the intersection of analytical chemistry, catalysis, materials science, and bio-inspired research is continuing to unfold.[6, 23–29] Particular interest was generated by the possibility of using cucurbiturils as vehicles in drug delivery and other pharmacological contexts.[30–36] Herein, we exploited a strategy for the phototriggered release of a model guest drug from cucurbit[7]uril (CB7) by means of controlling host–guest equilibria with a photoswitch. The cis–trans photoisomerization of azobenzene motifs is a frequently used relay mechanism for controlling supramolecular assemblies, including cucurbituril complexes.[7, 37–40] In this work, we build on a chalcone-flavylium system, which enabled the photoinduced switching between a non-charged (chalcone) and a charged guest species (flavylium ion), with the latter binding about three orders of magnitude stronger to CB7 than the former (see below). Hence, upon irradiation a competitive guest is formed, able to displace an initially complexed drug (3,5-dimethyl-1-aminoadamantane, also known as memantine, a widely prescribed Alzheimer’s drug) from the macrocycle (see Scheme 1). The only

Scheme 1. Coupling of a flavylum photoswitch with a memantine-CB7 host–guest equilibrium for the phototriggered release of the drug (memantine). The percentage numbers indicate the amounts of memantine in its complexed or free form under the chosen experimental conditions (see text).

[a] Dr. N. Basílio
Laboratório Associado para a Química Verde (LAQV)
Rede de Química e Tecnologia (REQUIMTE)
Departamento de Química, Faculdade de Ciências e Tecnologia
Universidade NOVA de Lisboa, 2829-516 Caparica (Portugal)
E-mail: nuno.basilio@fct.unl.pt

[b] Dr. U. Pischel
CIQSO - Center for Research in Sustainable Chemistry and Department of Chemistry
University of Huelva
Campus de El Carmen s/n, 21071 Huelva (Spain)
E-mail: uwe.pischel@diq.uhu.es

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precondition for the model drug is that its binding constant with CB7 should be ideally situated in the window defined by the ones for the chalcone and the flavylium ion.

In a first step we synthesized the water-soluble trans-chalcone 1a shown in Scheme 1, containing a sulfonate group (see Experimental Section). This compound interconverts with other species by means of pH and light stimuli showing the same intriguing chemical-network features (see Appendix 1 in the Supporting Information), as are known for other flavylium systems, such as the previously reported 4’-methoxyflavylium. \[41, 42\] For the purpose of this work, we focused on the photoinduced conversion between the trans-chalcone 1a and the flavylium ion 1b. Species 1a is stable at pH 3.15 in the dark. However, on irradiation at 366 nm for approximately 50 min (light intensity \(2.3 \times 10^{-7}\) Einstein min\(^{-1}\)) the flavylium ion 1b with its characteristic absorption band at 437 nm is formed quantitatively with a quantum yield of 0.08; see Figure 1. The identity of the flavylium ion 1b as photoproduct was confirmed by monitoring the irradiation of 1a by \(^1\)H NMR spectroscopy (see the Supporting Information). The system can be reverted to the trans-chalcone in a thermally activated process. Heating a solution of photogenerated 1b for about three hours at 70 °C yields back 1a \(k_{\text{obs}} = 4.0 \times 10^{-8}\) s\(^{-1}\) in 94% yield; see the Supporting Information. As the speciation in flavylium systems is known to depend very much on the proton concentration, photoirradiation at varying pH values was performed. This enabled the determination of an apparent \(K_a\) of 4.2 at the photostationary state (see the Supporting Information), thereby defining the requirement of pH ≤ 4 for our photoswitch. It is worth noting that the flavylium cation 1b can also form as the thermodynamic stable species in the dark at very acidic pH values \(\text{pK}_a\) ca. 1). However, such thermal conversion of 1a is very slow under our experimental conditions (half-life \(t_{1/2}\) ca. 100 days, see the Supporting Information). A large \(\Delta \text{pK}_a\) shift (ca. 3.2) between the photostationary state and the dark equilibrated solutions is critical for near quantitative photoconversion of the trans-chalcone into the flavylium ion.

Having established the chemical and functional basis of the photoswitch, the differential binding properties of 1a and 1b with CB7 were studied. On the one hand, the \(^1\)H NMR titration \[43\] of 1a with CB7 confirmed the predicted weak 1:1 binding (apparent \(K_a = 3.0 \times 10^4\) M\(^{-1}\)). Noteworthy, Na\(^+\) ions may be competitive CB7 binders at millimolar concentrations. \[44\] However, the accordingly corrected binding constant for 1a is very similar, \(K_a = 4.0 \times 10^4\) M\(^{-1}\); see Supporting Information. On the other hand, the UV/Vis absorption titration \[43\] of the photogenerated ion 1b (at pH 1) yielded a much larger 1:1 binding constant of \(K_a = 9.0 \times 10^4\) M\(^{-1}\); see Figure 2. This is straightforward rationalized by the significant ion-dipole interaction between the positively charged guest and one of the carbonyl portals of CB7. Such a feature is missing for 1a. The recording of \(^1\)H NMR spectra of 1b in the presence of CB7 corroborated the notion of the formation of a stable inclusion complex, which was accompanied by pronounced upfield shifts of the protons 4, 5, and 8 that are immersed in the CB7 cavity (see Figure 3). Similar observations have been made for the CB7-complexation of related flavylium systems, thereby providing solid ground for our interpretations. \[41, 45–48\]

According to the idea expressed in Scheme 1 we proceeded to the key experiment, testing the release of memantine from CB7 on transformation of the trans-chalcone 1a into the competitive binder 1b. For this purpose an acetate-buffered D\(_2\)O solution (pD = 4.4) containing optimized concentrations of CB7 \(1 \times 10^{-5}\) M, 1a \(1 \times 10^{-3}\) M, and memantine \(5 \times 10^{-4}\) M) was irradiated at 366 nm. The drug model memantine was chosen in a lower concentration to allow an efficient competition of the photogenerated flavylium ion 1b. The binding constant of memantine is known as \(K_m = 2.5 \times 10^4\) M\(^{-1}\) and, hence, it was expected that the flavylium ion \(K_a = 9.0 \times 10^4\) M\(^{-1}\) would displace the drug efficiently. \[46\] Indeed, in the \(^1\)H NMR spectrum the signals of the CB7-complexed memantine vanished on irradiation.

Figure 1. Spectral variations on irradiation (366 nm, \(t_i = 2.3 \times 10^{-7}\) Einstein min\(^{-1}\)) of the trans-chalcone 1a (1.9 \times 10^{-5}\) M) at pH 3.15 (see text and Supporting Information for the choice of optimal pH conditions). The inset shows the absorbance at 440 nm plotted against the irradiation time.

Figure 2. Spectral variations observed for 1b \(4.1 \times 10^{-5}\) M upon gradual addition of CB7 at pH 1 (to ensure the presence of the flavylium cation, which is stable under these conditions). The inset shows the absorbance variations registered at 424 nm plotted against the CB7 concentration. The fitting line corresponds to a 1:1 binding model.

\[41, 45–48\] These are not the final page numbers!
stabilization of the flavylium ion quantitative reversal of the situation is explained with a higher around 60% complexed memantine (Figure 4). The less than activated 1b situation was partially inverted due to the thermally acti-

vated 1a conversion, yielding back an amount of around 60% complexed memantine (Figure 4). The less than quantitative reversal of the situation is explained with a higher stabilization of the flavylium ion 1b in the CB7 complex compared to the free ion (see above). Under the chosen experimental conditions the equilibrium between 1b and 1a is characterized by approximately 50% flavylium ion, that is, only par-
tial memantine recombination occurred. The reversible inter-

conversion, if required by an application, can be achieved by optimizing the experimental conditions, for example, by using more dilute solutions. As an example, the mole fraction of the flavylium cation at the equilibrium is estimated to be 14% for a $1.9 \times 10^{-4}$ M solution of 1a in the presence of $5.0 \times 10^{-4}$ M CB7 at pH 3.9 (see the Supporting Information). It is worth noting that the design of reversibly addressable multicompo-

nent systems consisting of CB host–guest complexes and pho-
toresponsive switches is not a trivial task, because the pres-
ence of the host macrocycle often leads to a stabilization of the photoproduct. However, it is also important to note that, for drug delivery applications, reversibility is not a pre-re-

requirement at all.

In conclusion, we devised a method for the photocontrolled release of the memantine model drug from the cucurbit[7]uril macrocycle in water. The release is mediated by the formation of a strongly binding flavylium cation as competitor by irradiation of a weakly binding trans-chalcone as precursor. The herein demonstrated proof-of-principle could be expanded to other photoswitchable systems and different chemical environments.

**Experimental Section**

**General:** All solvents and chemicals employed for synthesis and for preparation of samples were of reagent or spectrophotometric grade and used as received. Millipore grade water was used. Cur-

curbit[7]uril was available from previous studies. The final pH of the solutions was measured with a Crison basic 20 + pH meter. UV/Vis absorption spectra were recorded with a Varian Cary 100 Bio or a Varian Cary 5000 spectrophotometer. NMR experiments were run on a Bruker AMX 400 instrument operating at 400 MHz (1H) and 101 MHz (13C).

**Synthesis:** 4’-Hydroxyacetophenone (1.0 g, 7.3 mmol) was treated with one equivalent of 1,4-butane sultone and one equivalent of sodium carbonate in 10 mL isopropanol and stirred under reflux overnight. After cooling to room temperature, the solution was filtered and the solid was carefully washed with methanol. The filtrate was concentrated by rotary evaporation and the product was precipitated by addition of diethyl ether. After filtration, further washing with diethyl ether, and drying in high-vacuum, 1.3 g (60% yield) of an off-white solid were obtained. The identity of the de-

sired 4’-(1-sulfo-4-butyloxy)acetophenone sodium salt was con-

firmed by $^1$H NMR spectroscopy. $^1$H NMR (400 MHz, D$_2$O): $\delta$ = 7.92 (d, $J$ = 8.6 Hz, 2 H), 6.99 (d, $J$ = 8.6 Hz, 2 H), 4.12 (t, $J$ = 5.5 Hz, 2 H), 2.90 (t, $J$ = 7.2 Hz, 2 H), 2.52 (s, 3 H), 1.93–1.78 ppm (m, 4 H). 4’-(1-Sulfo-4-butyloxy)acetophenone sodium salt (0.1 g, 0.34 mmol) and salicylaldehyde (0.04 g, 0.34 mmol) were dissolved in 0.4 mL methanol and the solution was cooled down to 0°C with an ice bath. After addition of 0.044 mL of 40% NaOH, the mixture was allowed to warm to room temperature and stirred overnight. The re-

action mixture was diluted in 5 mL of distilled water, neutralized with 1 M HCl, and extracted with diethyl ether. The aqueous phase was concentrated by evaporation and the crude product was puri-

fied by reverse phase (C18) column flash chromatography with gra-
dient elution from 100% H$_2$O to 70% H$_2$O/30% CH$_3$CN. After evap-
oration of the solvent and drying in high vacuum 1a was obtained as a yellowish solid (0.1 g, 74% yield). $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ = 8.03–7.92 (m, 3 H), 7.73 (t, $J$ = 15.8 Hz, 1 H), 7.57 (dd, 1 H), 7.19–

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Figure 3. $^1$H NMR spectra of (a) the memantine-CB7 complex, (b) 1a (1 $\times$ 10$^{-3}$ M), CB7 (1 $\times$ 10$^{-2}$ M) and memantine (5 $\times$ 10$^{-4}$ M) before irradiation, (c) the same after one hour of irradiation at 366 nm, (d) free memantine, and (e) the same as in (c) after standing overnight in the dark at 70°C. The spectra (a) and (d) were acquired in non-buffered D$_2$O and are shown for the sake of comparison. The remaining experiments were performed in 0.01 M acetate-buffered D$_2$O solution (pD = 4.4). The pH (pD) condition of these experiments was chosen to guarantee optimized photochromic transformation and thermal recovery in the presence of CB7. The somewhat higher pH (4 vs. 3.2 in the absence of CB7) was adjusted due to the known pK$_a$ shift of guests upon CB7 complexation.

Figure 4. $^1$H NMR spectra of (a) the memantine-CB7 complex, (b) 1a (1 $\times$ 10$^{-3}$ M), CB7 (1 $\times$ 10$^{-2}$ M) and memantine (5 $\times$ 10$^{-4}$ M) before irradiation, (c) the same after one hour of irradiation at 366 nm, (d) free memantine, and (e) the same as in (c) after standing overnight in the dark at 70°C. The spec-
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28. The CB7-binding constant of 1a is too small to be determined in the micromolar concentration regime applied in UV/Vis-absorption titrations. However, strong binders such as 1b are not feasible for NMR titrations, typically performed at millimolar concentrations.

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Communications

Lights on and action: Controlling a host–guest equilibrium with a flavylum-based photoswitch leads to the efficient light-triggered release of memantine, a widely prescribed Alzheimer’s drug. The supramolecular host system is a cucurbituril macrocycle, which binds preferably to a flavylum ion that is formed from a trans-chalcone in a photo-triggered reaction. Thereby the initially complexed memantine guest is competitively displaced.


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